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# Growth Control: p53, the Guardian Angel of Compensatory Proliferation

**Compensatory growth, or regeneration, is used to replace damaged tissue during animal development. Recent work has revealed a new role for *Drosophila* p53 in the compensatory proliferation of cells that are needed to repair damaged tissues, a role that requires the non-apoptotic function of the caspase protease Dronc.**

**Eric H. Baehrecke**

Organ and tissue size is regulated by cell proliferation, cell growth and cell death during animal development [1]. Remarkably, tissues with severe damage are capable of regeneration during development through a process of compensatory growth, resulting in tissues and structures of normal size and pattern [2]. Such damage is triggered by cellular insults, including DNA damage, that activate programmed cell death (apoptosis). p53 is a key regulator of the response to genotoxic stress, and its importance in suppressing tumor formation is underscored by its inactivation in many human cancers [3]. p53 responds to cellular insults either by arresting the cell cycle, so that DNA can be repaired, or by triggering apoptosis [4]. A recent study published in *Current Biology* by Wells *et al.* [5] implicates cell-death regulators, including p53, in compensatory proliferation and regeneration of damaged tissues during development of the fruit fly *Drosophila melanogaster*. The discovery of cell-death regulators that promote cell proliferation and regeneration provides a new twist in our understanding of the mechanisms controlling life and death decisions during animal development.

Compensatory cell proliferation is used to repair developing adult structures (imaginal discs) in *Drosophila* following the induction of cell death [6–8]. 'Undead cells' can be created in developing fly tissues by activating cell death while protecting against the demise of these cells with expression of p35, an inhibitor of caspase proteases. This results in overgrowth of tissues because of compensatory proliferation of cells that is associated with ectopic expression of the patterning regulators Wingless (Wg) and Decapentaplegic (Dpp). Additionally, Jun N-terminal kinase and the initiator caspase Dronc have been implicated in the regulation of overgrowth, but several mysteries about this tissue repair mechanism remain unsolved.

Wells *et al.* [5] recognized that the creation of large regions (compartments) of undead cells in fly wing imaginal discs causes a 3–4 day developmental delay during the third larval instar stage. In addition, wing imaginal discs that contain undead cells undergo growth arrest specifically during the third larval instar such that these tissues are smaller than those in control animals. Growth arrest in imaginal discs containing undead cells is transient, however, as these tissues can become nearly 50% larger than controls prior to the onset of pupariation. This prompted the authors to investigate the influence of undead compartments of cells on cell division in wing imaginal discs. They discovered that undead

cells arrest transiently at the G2 stage of the cell cycle, while other cells arrest permanently, and that these cell-cycle changes precede compensatory tissue growth. Furthermore, RNA levels of the Cdc25 homolog String (Stg) are reduced in undead cells, consistent with the function of *stg* in the regulation of mitosis [9]. Following the transient cell-cycle arrest, undead cells express *stg* RNA and proliferate at normal rates, even though paradoxically they express elevated levels of processed caspase-3. This results in tissue overgrowth that is associated with delayed larval development.

DNA damage causes G2 arrest, and Wells *et al.* [5] investigated whether genes involved in sensing DNA damage are also required for compensatory proliferation. Although the DNA-damage-sensing gene ataxia telangiectasia mutated (*atm*) and the downstream kinase gene *chk2* were not required for compensatory proliferation, p53 RNA was induced in undead cells. Furthermore, phosphorylated AMP-activated protein kinase, a protein that has recently been implicated in the regulation of p53 and G1 arrest in flies [10], was present at similar levels in imaginal discs with undead cells and in controls lacking undead cells. p53 mutants are sensitive to DNA damage that causes late larval and pupal lethality [11,12], and the authors found that p53 is required for several changes that are associated with undead cells. Significantly, mutations in p53 prevented cell-cycle arrest in both undead and neighboring cells and also blocked ectopic Wg expression and compensatory proliferation. In addition, p53 mutants with undead cells exhibited normal developmental timing.

Flies have a single p53 ortholog that is required for apoptosis after DNA damage [11,13,14], but p53 had not been previously implicated in the regulation of G2 arrest in *Drosophila*. Since the DNA-damage-sensing factors ATM and Chk2 were not required for compensatory proliferation, and elevated levels of cleaved caspase-3 were present in undead cells of p53 mutants, the authors investigated the previously recognized non-apoptotic role of the initiator caspase Dronc [6] in p53-regulated compensatory proliferation. Although the caspase inhibitor p35 blocks effector caspases, such as caspase-3 (Drice and Dcp-1 in flies), it does not inhibit Dronc [15], possibly explaining the presence of cleaved caspase-3 in undead cells. Significantly, Dronc mutants with undead cells did not exhibit overgrowth of imaginal discs and lacked ectopic expression of Wg, cleaved caspase-3 and p53 RNA.

The pro-apoptotic factors Reaper (Rpr) and Head involution defective (Hid) activate apoptosis by triggering the degradation of the *Drosophila* inhibitor of apoptosis 1 (Diap1) protein thereby enabling caspase activation [15]. The authors expressed Dronc and p35 in imaginal discs, and, although Diap1 protein was present, large overgrowth phenotypes still occurred in imaginal discs. Ectopic expression of Dronc and p35 resulted in elevation of p53 RNA, and p53 mutants suppress the Dronc-induced overgrowth phenotype in imaginal discs. These results indicate that Dronc is both necessary and sufficient to induce p53-dependent overgrowth in imaginal discs. p53 encodes a DNA-binding protein that directly regulates the transcription of *rpr* [13], and both p53 and Dronc function were required for elevated levels of *rpr* RNA in undead cells; this increase in *rpr* expression was triggered by expression of Hid and p35. Therefore, a feedback loop appears to exist in undead cells involving the cell-death regulators Rpr, Hid, Dronc and p53.

As previously mentioned, the presence of undead cells in imaginal discs triggers growth arrest, changes in expression of patterning factors, and compensatory proliferation [5–8]. The similarity between these responses and the events that occur during regeneration of damaged tissue in imaginal discs prompted Wells *et al.* [5] to investigate how p53 and Dronc influence blastema formation, the generation of a proliferating population of cells that is required for regeneration of damaged tissues [2,16,17]. Importantly, both p53 and Dronc mutants show a decrease in blastema formation compared with control wild-type animals. These results support a model that implicates p53 and Dronc — proteins that are often considered to be cell-death regulators in flies — in new roles as regulators of compensatory proliferation and blastema formation in the regeneration of tissues following the formation of undead cells.

The recognition that p53 and Dronc regulate compensatory proliferation and repair of damaged tissue is a significant advance in our understanding of animal development, but many important questions remain to be addressed. It is interesting that flies appear to have a mechanism to sense either damaged or underdeveloped tissue and prolong their development so that compensatory growth can occur. Although genes have been identified that coordinate nutritional status and growth [18], sensing the competence to form individual adult structures and coordinating this with global developmental transitions appears to be a very different process, and it will be important to determine the mechanisms underlying this complex problem. Will the mechanisms that regulate the repair of damaged tissues in flies, including roles for p53 and the caspase Dronc, be the same in all regenerating fly tissues, and be conserved in different organisms? Flies are clearly different from higher organisms; flies have a single p53 family member, while mammals have three members of this family. *Drosophila* p53 is more similar to mammalian p63 than p53 in the most highly conserved region, the DNA-binding domain [19], and, although fly p53 lacks an obvious sterile alpha motif (SAM) domain, which is present in p63, it is possible that mammalian p63 may possess some of the functions that are being discovered for fly p53.

It is intriguing that the protease Dronc regulates transcription of p53, and it will be critical to resolve whether Dronc cleaves

a transcription factor that regulates p53 transcription, or whether Dronc has an unknown biochemical activity that alters transcription in a protease-independent manner. It is not clear what happens to caspase substrates in undead cells, and, if caspase substrates are cleaved, whether or not they are completely degraded. Do undead cells maintain epithelial structures such as cell polarity and cell junctions, and could the presence or absence of these characteristics contribute to their capacity to regenerate a tissue? It is curious that undead cells do not appear to be present at earlier stages in development even though the factors that promote their formation are expressed days earlier, suggesting that unknown mechanisms may exist to protect imaginal cells. Finally, it is important to determine the growth signal that is produced by undead cells. Recent work indicates that *wg* function is not required for tissue overgrowth [20], and, while Dpp induction occurs in *wg* mutant imaginal discs, Wells *et al.* [5] show that Wg and Dpp targets are downregulated in undead cells. Clearly, much work is required to understand how decisions of life and death are regulated in the context of animal development, but for now this study provides a significant advance by showing that killers can serve as guardian angels that facilitate the repair of damaged tissues.

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# Nursing Behavior: Remembrance of Things Past

**Successful suckling is vital to the survival of mammalian newborns. In many mammals, nursing behavior is triggered by maternally derived odors. Such odors may also promote the learned association of odorant cues present in the environment during nursing.**

**Nirao M. Shah**

A mammalian newborn needs to nurse soon after birth, as it relies exclusively on milk from the mother for nourishment. In most mammals, the young navigate to the mammary glands without physical assistance from the mother and proceed to suckle effortlessly [1]. The complexity inherent in navigating to and grasping the nipple suggests that this innate response may benefit from learned associations that permit more efficient nursing [2]. Recent work in the rabbit [3,4], including work published in this issue of *Current Biology* [4], sheds light on learning promoted by cues that trigger suckling.

Newborns of many species display innate, species-specific behaviors to elicit food from the parents. For example, thrush nestlings present a wide open mouth — the gaping reaction — to their parent, who then deposits food into the oral cavity [5]. In this case, the nestlings gape in response to the particular visual profile displayed by adult thrushes. Mammalian newborns initiate suckling attempts in response to maternal cues. In many mammals, including rodents and rabbits, olfactory cues play an essential role in initiating nursing [1]. The response of the lactating mother is